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MATHEMATICAL WELFARE ASSESSMENT MODEL OF CHICKEN BREEDER FLOCKS

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Abstract

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The aim of the present study was to evaluate the mathematical welfare assessment model of New Hampshire breeder flocks reared under different production systems and to examine the effect of welfare improvement during the hot period by dietary supplementation with zinc and vitamin C. For this purpose, changes in poultry behaviour, blood corticosterone concentrations and some biochemical blood indices were determined.

Using a mathematical welfare assessment model, the integrated assessment of poultry welfare (PW) of breeders reared indoor on litter was PW=33.33 %. It was based upon statistically significant changes in different behavioral traits, higher blood corticosterone ($P<0.001$), cholesterol ($P<0.001$), glucose ($P<0.001$), triglycerides ($P<0.01$), compared to birds reared in the free-range system. Free range- reared breeders had a PW=60%.

The dietary supplementation of 35 mg/kg zinc and 250 mg/kg vitamin C contributed to reduction of the adverse effect of stressors in both rearing systems and to improvement of poultry welfare to PW= 60% in indoor-reared breeders and up to 80% in free range-reared breeders.

Key words: mathematical poultry welfare assessment model, chicken breeders, indoor on litter and free range systems, behaviour, corticosterone, biochemical blood parameters

Introduction

During the last years, reliable models and criteria for evaluation of poultry welfare under different production systems are extensively sought (De Mol et al., 2006; Forkman et al., 2007; Tuyttens et al., 2008; Linares and Martin, 2010). Often, in temperate climate regions, a number of environmental factors have a negative impact on poultry welfare: high ambient temperatures, very low or very high air humidity, constant exposure to ammonia concentrations close to allowances etc (Hocking at al., 2001; Sahin at al., 2002a,b; De Jong et al., 2002a,b; Sahin and Kucuk, 2003).

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One of the economically profitable approaches to stress reduction and welfare improvement in birds is the dietary supplementation with zinc and vitamin C. Bartlett and Smith (2003) and Sahin et al. (2005) believe that this alleviating effect is due to the lower concentrations of antioxidant minerals and vitamins as zinc and vitamin C**.** Birds are able to synthesize vitamin C, but this ability is inadequate under stress conditions such as low or high environmental temperatures, humidity, high productivity etc. (McDowell, 1989). Stress triggers an excessive production of free radicals (Halliwell and Gutteridge, 1989) namely oxidative stress (Sahin et al., 2002a, b; Lin et al., 2006). Zinc is known to play a key role in the endogenous antioxidant protection system. Bains (1996) and Frandson, (1986) reported a corticosterone-modulating effect of vitamin C via its involvement in the gluconeogenesis to enhance energy supply during stress. Thus, the negative effect of stress in poultry is reduced and their welfare – improved. The issues related to stress and physiological comfort in birds reared under different production systems are still a challenge.

Poultry welfare assessment uses integrated approaches based on different criteria. Platz at al. (2003); Dawkins (2003); Linares and Martin (2010) outlined that the natural behaviour of birds was the most relable welfare indicator. In the view of Gonzales-Esquerra and Leeson (2006); Lin et al. (2006); Mormède et al. (2007), along with behaviour alterations, poor welfare is accompanied with increased blood corticosterone, total cholesterol, blood glucose (Yanchev et al., 2007; Sahin et al., 2009) and triglycerides (Puvadolpirod and Thaxton, 2000 a,в; Popova–Ralcheva et al., 2002 a,в).

The purpose of this research is to assess the welfare of chicken breeders under different rearing systems (indoor on litter and free range system) and feed with diet supplemented with zinc and vitamin C, during the hot summer period.

Materials and Methods

The experiments were carried out with 48 weeks old New Hampshire breeders, (n=208) reared under two systems – indoor on litter (variant A) and free range system with sleep houses and walk yards (variant B).

The investigations were carried simultaneously during the hot summer period from June 21 to July 21 2010 at two locations: the Poultry Production Base at the Institute of Animal Sciences – Kostinbrod and at the Poultry farm of the Department of Animal Science, Agriculture University – Plovdiv. The sex ratio in the flocks was 1 rooster per 8 hens.

For variant A, two boxes of chickens (control and experimental) housed in the same premise were selected. Breeders were reared in groups of 52 birds on litter in-group boxes with a density of 4 hens/m^2 in one premise with natural ventilation. They were fed by round tubular feeders ensuring feeding and drinking widths of 4 and 3 cm respectively, as required by zoo hygienic norms (Ordinance 44/2006). For egg-laying, single-floor wooden nests with dimensions 30/30/40 cm were provided (4 hens in one nest).

In the free-range system (variant B), birds from each group were housed in sleeping pens and outdoor walking yards. Two groups of 52 birds each were formed (experimental and control). Each group was housed in identical sleeping pens (3.50/2.50/2.75 m) which were equipped with perches and two-floor wooden nests (30/30/40 cm). The light intensity coefficient was 1:10. In the bottom of the southern wall of pens, there was a 30/40 cm rectangular opening for outdoor access. Each yard was 9.20/24 m with perennial broadleaf trees in the middle. Yards were provided with two rows of tubular feeders and with watering troughs ensuring feeding and drinking widths of 10 and 3 cm, respectively as required by zoohygienic norms for this category of birds (Ordinance 44/2006).

Throughout the experimental period, control birds from both rearing systems (A and B) were fed freely with the same compound feed according to birds' category (NRC, 1994).

Birds from variant A and B experimental groups (Zn + Vitamin C) received the same compound feed supplemented with 100 mg/kg Zinteral 35 (Lohmann Animal Health, Cuxhaven, Germany), containing 35 mg zinc/kg as zinc oxide together with 250 mg vitamin C (L-acidum ascorbicum, CSPC Weisheng Pharmaceutical, SHIJIAZHUANG Co. Ltd) per kg feed.

Microclimatic conditions were determined by routine methods. The temperature and the relative humidity of air were measured with a weekly thermohygrograph; the velocity of the air motion – with a catathermometer, the light intensity – with a electronic luxmeter, the concentration of ammonia – with indicator tubes and Drager ammonia sensor and calculated in ppm.

Blood samples for analysis were obtained from randomly selected six birds from each group on the 21st of July from v. subcutanea ulnaris in sterile vacuumtainers. The duration of each manipulation was not longer than 2 min i.e. lower than the maximum time that did not influence plasma corticosterone concentrations in birds, Lagadic et al. (1990). The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min.

The behaviour of parent flocks was recorded with a video camera for 12 hours during 4 consecutive days – 17th , 18 th, 19 th and 20 th July. Based on the recordings were prepared ethogrammes as per Wojcik and Filus (1997). During the ethological study was counted the number of birds engaged in specific forms of behaviour: ingestive (ingestion of water or food), gregarious (moving, resting, egg-laying, dust bathing and feather cleaning), sexual and agonistic behaviour as per Popova-Ralcheva et al. (2002a).

The plasma coricosterone levels were assayed with immunoenzymatic ELISA kit (Corticosterone ELISA RE52211, IBL Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany) in the Laboratory of Innate Resistance Investigation at the Veterinary Genetics and Breeding Unit, Department of General Animal Breeding, Faculty of Veterinary Medicine – Stara Zagora.

Blood biochemical indices – glucose, cholesterol, creatinine, urea, total protein and triglycerides were determined with an automated biochemical analyzer "Cobas mira" at an accredited biochemical lab in the Diagnostic and Consultation Medical Centre "St. George"- Plovdiv.

The welfare assessment score was calculated by a modification of the system of Bozakova (2004) and Bozakova et al. (2011) based on the scientific concept of animal welfare of the UK Farm Animal Welfare Council (FAWC, 1995). It included the so-called „five freedoms" guaranteeing poultry welfare, which are given a specific score: 3; 2; 1 and 0, depending on expression of a particular behavior, plasma corticosterone concentrations and some blood biochemical indices under different production systems.

The utmost importance in welfare assessment was attributed to the freedom of pain, injury and disease (F_3). The final score was obtained as a sum of numerical expressions of all freedoms and compared to the maximum possible score of 15, expressed in percentage.

The integrated assessment of poultry welfare (PW) in the four groups was calculated according to the formula:

$$PW=\frac{a.(F_1+F_2+F_3+F_4+F_5)}{15}.100\%$$, where:

F_1, F_2, F_3, F_4, F_5 are scores for each of the five freedoms in birds.

They can take values of 0, 1, 2 or 3 according to the manifestation of the specific freedom in a

specific situation; a=1, provided that the score of the freedom from pain, injury and disease is not zero ($F_3 \neq 0$);

a=0 if $F_3=0$, as welfare is not possible if the organism of the bird could not adapt to the production system and this entails a progressive disease (Broom, 2006).

Statistical processing of the results was performed by means of one-way ANOVA using the GraphPad InStat 3.06 software to determine the level of significance among mean values. raphPad InStat 3,06 software at level of significance $P<0.05$.

Results

The data for the microclimatic indicators of New Hampshire breeder flocks reared under two production systems are given in Table 1.

Comparing microclimatic parameters in the birds' living area with the parameters of veterinary requirements for animal breeding facilities, Ordinance 44/2006, it was determined that the average ambient temperature in the indoor production system – 31.00±0.60^{0}C, i.e. substantially higher that the allowances of 18-25^{0}C for this category birds. Ammonia concentrations were 14.07±0.54 ppm, i.e. close to the maximum allowed limit of 15 ppm. The other parameters were within the reference range.

At the same time, in the free-range production system, the average air temperature for the study period was 28.86±0.63, again higher than the reference range, while only traces of ammonia were present. The lighting intensity in the outdoor system was considerably higher than that birds were accustomed. The other parameters were within the reference range.

Observing the changes in the behaviour of the breeder flocks, reared under different production systems (Table 2) significant differences between control and supplemented (Zn + vitamin C) groups, as well as between control birds reared under different systems was established.

During the hot summer period, experimental chickens (Zn + vitamin C), reared indoor on litter (Variant A) exhibited more intensive dust bathing ($P<0.01$) and sexual ($P<0.05$) behavior, along with less aggression ($P<0.05$) compared to controls.

In the free range rearing system (Variant B) lying ($P<0.05$), dust bathing ($P<0.05$) and mating ($P<0.05$) birds supplemented with zinc and vitamin C were more numerous than controls, but there were less moving ($P<0.001$) and aggressive birds ($P<0.05$), compared to the non-supplemented group.

The most consistent differences were observed in the behaviour of control birds under both rearing systems. In the free-range system, there were more feeding ($P<0.05$), egg lying ($P<0.001$), feath-

Table 1
Microclimate parameters for the New Hampshire breeders, reared under different production systems during the hot period

Rearing system	Ambient temperature, ^{o}C	Air humidity, %	Air velocity, m/s	NH_3, ppm	Light intensity, lx
Indoor on litter	31.00±0.60	59.27±1.38	0.8±0.03	14.07±0.54	31.25±1.74
Free range system - sleep houses and walk yards	28.86±0.63	52.63±0.75	1.17±0.14	traces	458.33±72.60
Reference values*	18 - 25	50 – 70	0.2 - 0.5	< 15	30 – 60

*Reference values as per Ordinance 44/2006

er cleaning (P<0.05), dust bathing (P<0.001) and mating (P<0.01) birds compared to the respective numbers in the flock, reared indoor. At the same time, there were les drinking (P<0.001), resting (P<0.01) and aggressive (P<0.05) birds compared to the indoor flock.

Blood corticosterone concentrations in birds reared either indoor on litter or in a free-range system, or supplemented or not with Zn and vitamin C (Table 3), showed significantly lower (P<0.01) hormonal levels in experimental birds compared to the controls. Blood corticosterone in control birds reared in the free-range system were also significantly lower than those reared indoor (P<0.01).

The tested blood biochemical parameters (Table 3) exhibited lower glucose concentrations in birds

Table 2

Number of New Hampshire breeders, supplemented or not with zinc and vitamin C, and exhibiting a specific type of behaviour reared under different production systems (mean ±SEM, n=6)

	Indoor on litter				Free range system			
Behaviour	Control group	%	Zn + vitamin C group	%	Control group	%	Zn + vitamin C group	%
Feeding	8.05±1.14	15.48	8.5±1.25	16.35	10.59±0.91$^{\#}$	20.36	9.77±0.68	18.79
Drinking	12.96±0.59	24.92	12.14±0.53	23.35	8.59±0.54$^{\#\#\#}$	16.52	8.55±0.55	16.44
Egg-laying	2.09±0.34	4.02	2.41±0.36	4.63	4.50±0.44$^{\#\#\#}$	8.65	5.82±0.60*	11.19
Moving	9.18±0.48	17.65	8.00±0.35	15.38	8.59±0.27	16.52	6.68±0.33***	12.85
Resting	7.23±0.76	13.90	7.55±0.81	14.52	4.68±0.67$^{\#\#}$	9.00	5.14±0.62	9.88
Feather cleaning	2.68±0.23	5.15	2.95±0.25	5.67	3.41±0.27$^{\#}$	6.56	3.64±0.30	7.00
Dust bathing	0.73±0.15	1.40	1.32±0.19**	2.54	1.73±0.16$^{\#\#\#}$	3.33	2.23±0.16*	4.29
Aggression	5.68±0.36	10.92	4.82±0.28*	9.27	4.86±0.28 $^{\#}$	9.35	4.00±0.23*	7.69
Sexual behaviour	3.45±0.20	6.63	4.14±0.27*	7.96	4.32± 0.22$^{\#\#}$	8.31	5.00±0.27*	9.61

*P<0.05; **P<0.01 ***P<0.001: statistically significant difference between control and experimental (Zn+vitamin C) groups;
$^{\#}$P<0.05; $^{\#\#}$P<0.01 $^{\#\#}$P<0.001: statistically significant difference between control groups under different rearing systems

Table 3

Blood corticosterone levels and biochemical indices in New Hampshire breeders reared under different production systems and supplemented or not with zinc and vitamin C for one month during the hot summer period (mean ±SEM, n=6)

	Indoor on litter		Free range system	
Parameters	Control group	Zn + vitamin C group	Control group	Zn + vitamin C group
Corticosterone, nmol/L	129.48±5.64	104.69±3.03**	87.92±5.61$^{\#\#}$	62.83±5.17**
Glucose, mmol/L	10.20±0.32	8.96±0.24**	7.90±0.21$^{\#\#\#}$	7.28±0.23*
Total cholesterol, mmol/L	4.64±0.19	3.77±0.25**	3.32±0.12$^{\#\#\#}$	2.84 ± 0.16*
Urea, mmol/L	0.98±0.01	0.87±0.003*	0.87±0.003$^{\#}$	0.85±0.006
Creatinine, μmol/L	27.35±1.12	26.58±0.67	25.67±0.49	24.67±0.71
Total protein, g/L	58.93±0.68	56.90±1.30	57.16±0.92	54.95±1.40
Triglycerides, mmol/L	0.56±0.02	0.52±0.01*	0.48±0.02$^{\#\#}$	0.41±0.02*

*P<0.05; **P<0.01: statistically significant difference between control and experimental (Zn + vitamin C) groups;
$^{\#}$P<0.05; $^{\#\#}$P<0.01 $^{\#\#}$P<0.001: statistically significant difference between control groups under different rearing systems

supplemented with zinc and vitamin C than controls (P<0.01 in the indoor system; P<0.05 in the free-range system), total cholesterol (P<0.01 in the indoor system; P<0.05 in the free-range system), and triglycerides (P<0.05 in both systems). Freely reared control chickens had lower blood glucose (P<0.001), total cholesterol (P<0.001), urea (P<0.05) and triglycerides (P<0.01) compared to the control flock reared indoor on litter.

Table 4 presents the integrated evaluation of welfare of New Hampshire breeder flocks reared either indoor on litter or in a free-range system, supplemented with (Zn and vitamin C) during the hot summer period.

Discussion

During the summer in temperate climate zones (June 21 – July 21), there were several deviations from the microclimatic allowances in chicken breeder flocks reared in two production systems: high ambient temperature (> 28^0C) in the outdoor system, and very high air temperature (31^0C), ammonia concentrations close to maximum allowed ones in the indoor system. These environmental stressors induced higher blood corticosterone concentrations (P<0.01) in control breeders reared indoor on litter than in birds under free-range system. Comparable data were reported by Sahin and

Table 4
Welfare assessment scores of New Hampshire breeders reared under different production systems and supplemented or not with zinc and vitamin C during the hot summer period

Poultry welfare assessment		Indoor on litter		Free range system	
Freedom	Degree	Control group	Zn + vitamin C group	Control group	Zn+vitamin C group
Freedom from thirst and hunger-F_1	0-excessive thirst and hunger 1-limited thirst and hunger 2-lack of thirst and hunger 3-excessive feeding and drinking	1	2	1	2
Freedom from discomfort-F_2	0 -excessive discomfort 1-limited discomfort 2-limited comfort 3- full comfort	1	2	2	3
Freedom from pain, injury disease-F_3	0-exhausting disease 1-limited disease 2-occasional pain and injury 3-lack of pain and injury	2	2	2	2
Freedom to express normal behaviour-F_4	0-behaviour disturbance 1-limited behaviour expression 2-moderate expression 3-full expression	1	2	2	3
Freedom from fear and distress - F_5	0-fear and distress 1-limited fear and distress 2-partial freedom 3-full freedom	0	1	2	2
Total score		5	9	9	12
Poultry welfare assessment, %		33.33	60	60	80

Kucuk (2003); Sahin et al. (2004); Gonzales-Esquerra and Leeson (2006); Lin et al. (2006) under the influence of environmental stress factors.

These stressors reflected upon the behavior of birds, both control and supplemented with Zn and vitamin C. A lower number of feeding ($P<0.05$), egg lying ($P<0.001$), feather cleaning ($P<0.05$), dust bathing ($P<0.001$) and mating ($P<0.01$) control breeders were observed indoor on litter compared to control birds under free-range system. At the same time, indoor on litter poultry drinking was more intensive ($P<0.001$), as well as resting ($P<0.01$) and aggression acts ($P<0.05$).

The increased aggression in the flock and blood corticosterone could be reliable indicators for the level of stress and therefore, could indicate a poor welfare (Popova-Ralcheva et al., 2002 a, b; Mormède et al., 2007). Hocking et al. (2001) and Broom et al. (2001) report similar data. These data are further supported by the blood biochemical profiles showing higher serum glucose ($P<0.001$), total cholesterol ($P<0.001$) and triglycerides ($P<0.01$), compared to control free range- reared birds. Our findings correspond to evidence provided by Donkoh (1989) and Sahin et al. (2002a,b) for increase in these parameters in broilers, Japanese quails and layer hens resulting from heat stress.

Therefore, the freedoms F_1, F_2, F_4 were scored with 1 point instead of the maximum 3 (Table 4.). The score for the freedom from pain, injury and disease (F_3) was 2. The score for the freedom from fear and distress (F_5) was zero, due to high blood corticosterone, glucose, cholesterol and triglycerides as compared to control freely reared breeders. That is why the integrated poultry welfare assessment in control birds, reared indoor, was PW =33.33%.

Indoor-reared breeders, supplemented with Zn and vitamin C, received a higher score for the freedoms: F_1, F_2, F_3, and F_4 with 2 points. The freedom from thirst and hunger (F_1) was 2 as their needs from both antioxidants were better satisfied under stress. The freedom from fear and distress (F_5) was scored with 1 because of fewer aggression acts ($P<0.05$), lower blood levels of corticosterone ($P<0.01$), glucose ($P<0.01$), total cholesterol ($P<0.01$) and triglycerides ($P<0.05$) compared to controls. A similar reduction in these indices under the influence of zinc supplementation in conditions of heat stress was reported by Kucuk et al. (2003), Yanchev et al. (2007) and Sahin et al. (2009)**,** Donkoh (1989) and Sahin et al. (2002a,b) demonstrated reduction in these parameters in broilers, Japanese quails and layer hens after supplementation of antioxidant vitamins in conditions of heat stress.

The observed effect on corticosterone concentrations could be attributed to the antioxidant and anti-stress effect of zinc and vitamin C. Being a co-factor of essential antioxidant enzymes – Cu/Zn superoxide dismutase and inhibiting NADPH-dependent lipid peroxidation (Prasad, 1997; Prasad and Kucuk, 2002), zinc limits the excessive secretion of corticosterone tightly linked to stress and anxiety in birds. In addition, Onderci et al. (2003) reported that supplemental zinc increased serum vitamin C, which, according to Frandson (1986) decreases blood corticosterone by including it in gluconeogenesis during stress. Thus, Zn and vitamin C supplementation contributes to alleviation of ecological stress and more active sexual ($P<0.05$) and dust bathing behavior ($P<0.01$) in experimental birds, indicating a better level of welfare compared to controls. The integrated welfare assessment in supplemented breeders reared indoor was PW = 60%, i.e. higher than that in controls.

Ambient temperatures for control birds under free-range system were not so extreme and therefore, there were significantly more feeding, egg-lying, feather cleaning, dust bathing and mating birds, but less drinking, resting and aggressive breeders compared to respective controls reared indoor. This confirmed the higher welfare of the birds under free range rearing. In poultry, improved welfare is manifested with increased time spent in stretching,

feather cleaning and dust bathing (Sherwin and Kelland, 1998). Similarly Stoyanchev et al. (2006) believe that dust bathing in turkeys is a sign of utmost comfort. In addition, the lower blood corticosterone, glucose, total cholesterol and triglycerides in free range- reared birds than in indoor-reared birds, made us score the freedoms: F_2, F_3, F_4 and F_5 with 2 points. The Freedom from thirst and hunger (F_1) was scored with 1 point (Tabl. 4). The overall welfare assessment in control free range-reared breeders was PW=60%, higher than that in indoor-reared breeders.

The breeders reared under free-range system and supplemented with Zn + vitamin C exhibited statistically significantly more intensive dust bathing and sexual behaviour than controls, but less moving and aggressive acts. The blood corticosterone, glucose, total cholesterol and triglyceride concentrations were also considerably lower. The calmer and more purposeful behaviour of birds could be attributed to the antioxidant effect of zinc (Sahin et al., 2002a; Sahin et al. 2002b; Lin et al., 2006). Our data confirm the report of Jones et al. (1996) that the preliminary addition of vitamin C (24 h before the stressors) was accompanied with less panic and calmer behavior in Japanese quails. This effect was attributed to the corticosterone and fearfulness reducing effects of vitamin C in birds (Satterlee et al., 1993; Jones et al., 1996). This way, both supplements (Zn + vitamin C) act synergically in the reduction of temperature stress and contribute to the better welfare of birds.

Therefore, in this group, F_1, F_3 and F_5 were scored with 2 points and F_2 and F_4 was scored with 3 (Table 4). This way, the integrated welfare assessment in this group was the highest of all studied groups –PW= 80%.

Conclusions

During the hot summer period New Hampshire breeders reared indoor on litter were under the adverse influence of high ambient temperature and elevated ammonia concentrations, which stimulated the release of corticosterone, cholesterol, glucose and triglycerides in blood. Considering the more frequent aggression acts and the lower number of feeding, egg-lying, feather cleaning and dust bathing birds, the integrated poultry welfare assessment in this group was PW=33.33 %.

In the free range reared control flock, the number of feeding, egg-lying, feather cleaning, dust bathing and sexually active birds, as well as the fewer aggression acts, lower average corticosterone cholesterol, glucose and triglycerides in blood compared to the indoor-reared flock, the integrated welfare assessment was PW=60% .

In breeder flocks reared either indoor or in a free-range system, the supplementation of the feed with 35 mg/kg zinc (Zinteral) together with 250 mg vitamin C contributed to reduction of ecological stress and improved welfare, manifested through more frequent dust bathing, more frequent sexual acts, and lower aggression, blood corticosterone, cholesterol, glucose and triglycerides. On this basis, the integrated welfare assessment scores were higher than control birds: PW= 60% in breeders reared indoor on litter and PW= 80% in free range-reared breeders.

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